XXII. "*On the Tracheal System of Simulium.*" By Thomas Harold Taylor, M.A., communicated by Prof. Louis Compton Miall, F.R.S.

[Read October 1st, 1902.]

The tracheal system of the larva of Simulium (fig. 1) consists of a pair of longitudinal trunks running from the thorax to the posterior end of the abdomen. The two trunks are connected by dorsal commissures (fig. 1, *d*). Of these, there are three in the thorax, one between the pro- and mesothorax, one in the mesothorax, and one in the metathorax. There is also a dorsal commissure in the tracheal system of the head. Anteriorly the trunk ends abruptly between the pro- and mesothorax. Posteriorly it ends in the 8th abdominal segment, where it divides into a dorsal and a ventral branch. From each junction between two segments the tracheal trunk passes sharply downwards and then gradually rises to the junction next behind (see fig. 1). Near the lowest point of the curve arises the branch which supplies the segment (fig. 1, *v*). These segmental branches are present in the metathorax and each of the seven following segments. Each passes ventrally from its point of origin and divides into a descending and an ascending branch. At, or near the point of division there arises a fine branch, the initial thread, which runs towards the skin and ends in a spiracle. The last initial thread arises direct from the longitudinal trunk, and not from the segmental branch (fig. 1, *e*). The initial threads are solid and devoid of spiral marking.

Between the pro- and mesothorax, the longitudinal trunk gives off a slender branch which passes dorsally as the anterior dorsal commissure already mentioned. The trunk then bends sharply downwards, and after a short course divides into two branches which supply the head, pro- and mesothorax. At this point arises an initial thread, which is connected with the anterior thoracic spiracle. That part of the descending trachea from which the branches arise, and to which the initial thread is attached, may, from its ultimate fate, be conveniently described as the *spiracle-chamber* (see fig. 2). The corresponding part of the ventral

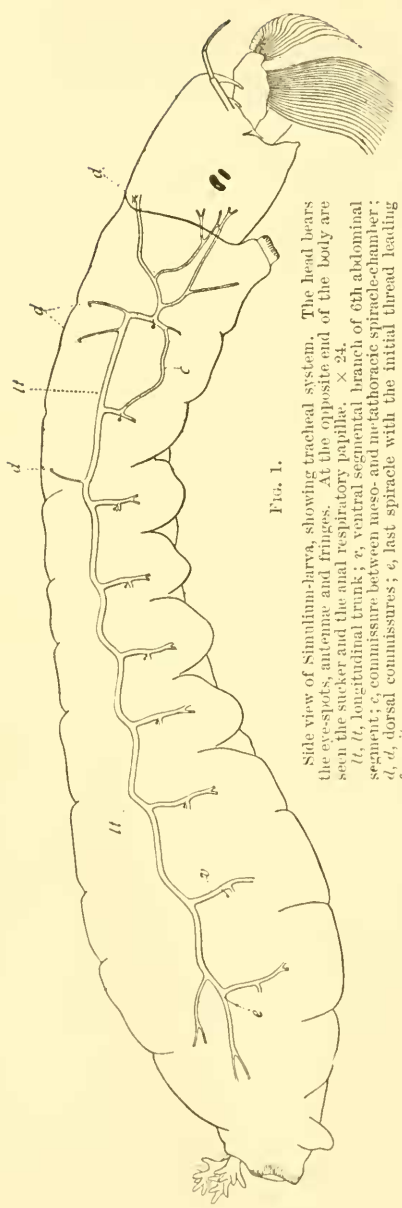


Fig. 1.

Side view of Simulium-larva, showing tracheal system. The head bears the eye-spots, antennæ and fringes. At the opposite end of the body are seen the sucker and the anal respiratory papillæ. × 24.
*l*, *l*, longitudinal trunk; *e*, ventral segmental branch of 6th abdominal segment; *c*, commissure between meso- and metathoracic spiracle-chamber; *d*, *d*, dorsal commissures; *e*, last spiracle with the initial thread leading from it.

branch in the metathorax may be similarly named. The position of the anterior thoracic spiracle is somewhat difficult to determine, but can be inferred from the following considerations. In old larvæ the developing pupal gill obscures the junction between the pro- and mesothorax. In larvæ in which the rudiment of the gill is beginning to develop a vertical muscle marking the intersegmental junction can be readily made out (fig. 2, *vm*). The spiracle is situated posterior to the muscle, and is therefore mesothoracic in position. Of the two branches which are given off from the spiracle - chamber one is anterior and the other posterior. Each again forks into a dorsal and a ventral branch. The dorsal member of the posterior branch unites with the succeeding metathoracic segmental branch (fig. 1, *c*). Each member of the anterior branch again divides, so that there are four tracheæ passing forwards, which may be numbered from above downwards the 1st, 2nd, 3rd and 4th. The 4th supplies the prothorax and its foot, the other

three the head. The 1st unites with the corresponding trachea of the other side by the head-commissure already mentioned. The 2nd and 3rd branches of the same side are connected together in the head by a short vertical trachea.

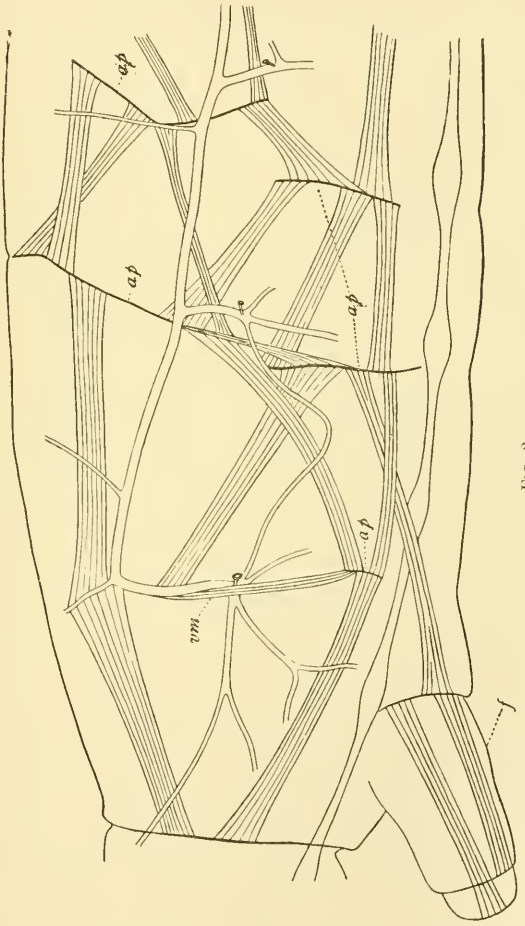


Fig. 2.

Thorax of Simulium-larva as a transparent object in side view. The muscles, tracheæ and nerve-cord are shown. The mesothoracic, metathoracic and 1st abdominal spiracles are seen. The initial threads from the spiracles proceed to the spiracle-ampullæ. × 75.
*vm*, vertical muscle marking junction between pro- and mesothorax; *f*, prothoracic foot; *ap*, apodemes marking junctions of segments

The tracheal system is renewed at each moult, the old one being cast with the skin. While the new tracheæ are developing, they enclose the old ones. It is evident that the new initial threads, which eventually become solid, must at this time be hollow. As the larva gradually disengages

the old cuticle from its body, the tracheæ, which are attached to it by means of initial threads are at the same time withdrawn through the new spiracles. To permit of this separation of the old tracheal system from the body, the tracheal trunks break across at certain points, which have a segmental arrangement. These tracheal junctions occur immediately posterior to the origin of the ventral segmental branches. At this point the spiral thickening of the intima is wanting. Junctions also occur on the transverse commissures. Two thickened annuli of chitin in the centre of the commissure replace the spiral intima, and the commissure snaps across between them. The withdrawal of the old tracheæ through the narrow opening afforded by the spiracle no doubt serves to squeeze the air from the old tracheæ into the new ones. Some air is, however, left sticking to the old collapsed tracheæ, as an examination of cast larval skins readily shows.

The spiracles are simple oval thickenings of the cuticle, and conspicuous from their black colour. The two thoracic and seven abdominal spiracles can be readily seen on the surface of the body with a low power. The mesothoracic spiracle is the largest of the series; the collapsed tracheæ which are pulled out through this spiracle from the head and thorax are much more numerous than those which are withdrawn from any of the other spiracles.

The tracheal system of the pupa (fig. 3) is modelled on that of the larva. The longitudinal trunks are connected by three transverse commissures in the thorax (fig. 3, *d*). Each trunk gives off in the abdominal segments 1—7 a ventral segmental branch, which divides into an ascending and a descending trachea. At their point of separation a slender initial thread passes to the anterior margin of the segment near which the spiracle is situated. The initial threads, like those of the larva, are solid and devoid of spiral markings. The spiracles are simply depressions of the skin. The spiracle of the first abdominal segment is concealed beneath the wing-sheath.

In the thorax the two pairs of spiracles have doubtless the same position as they have in the larva, but proof is difficult to obtain. The metathoracic spiracle is concealed beneath the wing-sheath. It is a wide, funnel-shaped depression of the cuticle, and is connected with the spiracle-chamber by means of a short thick initial thread. The

spiracle-chamber is connected with the longitudinal trunk by means of a short segmental branch, and the following branches lead from it: (1) a longitudinal commissure with the mesothoracic spiracle - chamber (fig. 3, *c*); about the middle of its course it gives off a trachea which runs in the sheath of the mesothoracic leg; (2) an ascending trachea which divides into a brush of tracheolæ; (3) a descending trachea which forks; (4) a trachea to the sheath of the metathoracic leg.

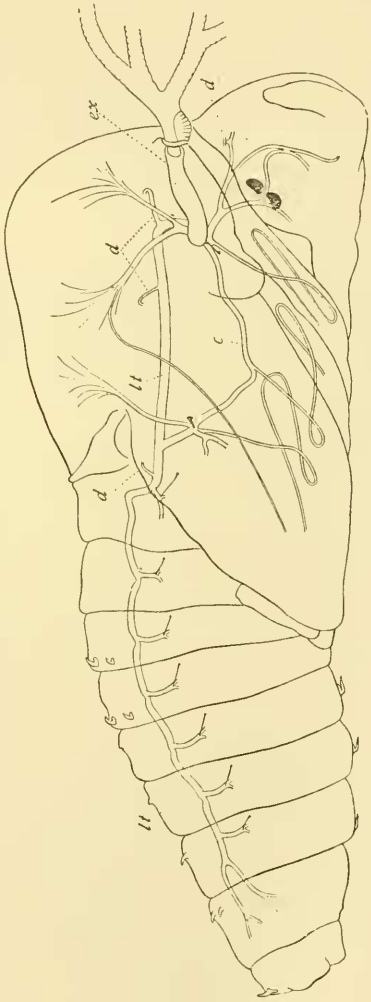

Fig. 3.

Pupa of Simulium in side view, the tracheal system being seen through the body-wall. The meso- and metathoracic and abdominal spiracles with their initial threads are shown in black. The filaments of the gill are cut short. × 24. *tt*, *tt*, longitudinal trunk; *c*, commissure between meso- and metathoracic spiracle-chamber; *d*, *d*, dorsal commissures; *ex*, tracheal extension.

The mesothoracic spiracle can be found most readily when the larval skin is being disengaged from the body of the young pupa. The old tracheæ of the head and part of the thorax are then withdrawn through the spiracle in question. It is situated a short distance behind the base of the gill. It becomes closed when the tracheæ are cast and the initial thread becomes solid. The spiracle-chamber gives off several tracheal trunks. Of these the longitudinal trunk and the commissure with the metathoracic spiracle-chamber have been already

mentioned. Of the remaining three branches, two have the same relations as the corresponding tracheæ in the larva. One supplies the sheath of the prothoracic leg; the other the head and fore part of the thorax. This last trachea divides into four branches which may be numbered as in the larva. The 1st is connected by a commissure with the corresponding trachea of the other side. The 2nd and 3rd branches of the same side are connected together as in the larva. The last trunk leading from the spiracle-chamber is met with in the pupa for the first time. It runs dorsalwards, and after a short course divides into an anterior and a posterior branch. Each of these breaks up into a copious brush of fine tracheolæ which supply the dorsal region of the thorax. From the posterior branch a slender trachea descends to supply the wing-sheath. The spiracle-chamber is put into communication with the pupal gill by means of a stout tracheal trunk which may be called the *tracheal extension* (fig. 3, *ex*).

The pupal gills of Simulium have been described by Vogler and others. They are paired, and carried on the fore part of the thorax. Each gill consists of a stem (fig. 3) which is short and thick, and of two (*S. latipes*) or four (*S. reptans*) branches, each of which divides into two long slender filaments. The stem projects forwards, and the filaments of each side lie nearly in one vertical plane. The filaments are transversely wrinkled, and taper gradually to a point. When viewed by reflected light under a low power of the microscope, the gill has a silvery appearance, but appears quite black and opaque when seen by transmitted light. After the gill is treated with caustic potash, transparent patches appear upon the filaments when viewed by transmitted light. These gradually enlarge and coalesce until the whole gill becomes transparent. The appearance of the gill is now dull by reflected light, and transparent by transmitted light. A similar result follows on the treatment of the gill with strong alcohol. By thus treating the gill with potash and with alcohol we learn that the filament is hollow, and that the wall is composed of a thick chitinous cuticle which resists potash solution. The chitinous cuticle is composed of two layers, a thin superficial and a thick deep stratum (figs. 4 and 5, *ss*, *ds*). The deep stratum is apparently quite homogeneous; it is very refractive, and stains readily with eosin and other dyes.

Separating the superficial from the deep stratum is a space about as wide as the deep stratum. This space is filled with air. It is to the presence of air in the chitinous

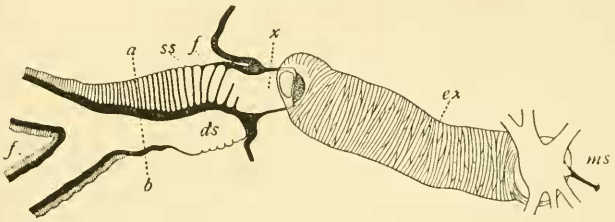


Fig. 4.

Tracheal extension and base of pupal gill. The gill-base is shown in vertical section the tracheal extension in surface view from the inner side. × 75.
*x*, communication between gill and tracheal extension; *f f*, chitinous fibrillæ; *ds*, deep stratum of wall of gill; *ss*, superficial do.; *ex*, tracheal extension; *ms*, meso-thoracic spiracle, with initial thread leading from the spiracle to the spiracle-chamber. The line *a b* indicates the plane of the transverse section (fig. 5).

wall that the silvery appearance of the gill when viewed by reflected light is due. Traversing this air-containing space and connecting the superficial with the deep stratum, are numerous delicate fibrillæ (figs. 4 and 5, *f*). These fibrillæ are simple at their deep ends, but tend to branch as they pass towards the superficial layer. They resist the action of hot caustic potash, and are probably chitinous like the rest of the wall. When the surface of a cleared filament is viewed by transmitted light, the points where the fibrillæ meet the superficial layer appear as bright dots which simulate pores. No apertures, however, occur in the wall of the gill.

The gill-stem differs from the rest of the gill in the arrangement of its chitinous layers. Four external surfaces may be distinguished: an upper, a lower, one turned towards the axis of the body, and another turned

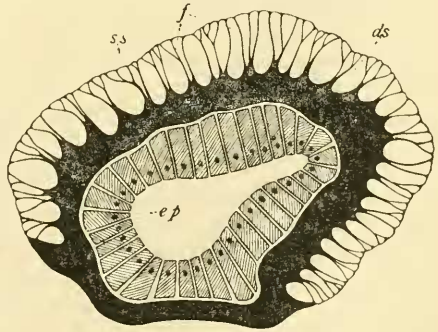


Fig. 5.

Transverse section of pupal gill, while still within the larval skin. × 300.
*vw*, compact ventral wall; *ss*, superficial stratum; *ds*, deep stratum; *ep*, epithelium; *f*, branched chitinous fibrillæ.

away from it. It is only on the upper wall of the gill-stem that the air-containing space is well developed. On the other surfaces the thin superficial layer becomes closely adherent to the deep layer, and the wall is rendered compact. As we approach the base of the gill-stem the contained air-space enlarges and the fibrillæ lengthen. This thickening of the dorsal wall of the gill-stem takes place at the expense of the internal cavity, which is consequently much reduced and perhaps quite obliterated. At the place of insertion of the gill the fibrillæ become fewer and farther apart, and finally disappear altogether. The air-containing space is now practically a tube excavated in the thickness of the cuticle of the dorsal wall of the gill.

The air-tube now leaves the gill, and enters the cavity of the thorax, in which it lies free. After a short course it ends abruptly by opening into a tracheal trunk (fig. 4, *x*). This tracheal trunk is the tracheal extension already mentioned. By means of this air-tube there is direct continuity between the air in the gill and the air in the tracheal system. Each gill has its own tracheal extension, by which it communicates with the mesothoracic spiracle-chamber of its own side. The tracheal extension (fig. 4, *ex*) is a wide tube lying near the wall of the thorax. The communication between the tracheal extension and the air-tube from the gill is not easily demonstrated, but its existence may be inferred from the following experiment. If a living pupa with entire gills be placed in strong alcohol, the air is rapidly driven out of the filaments, which consequently lose their silvery appearance. On replacing the alcohol with water, air soon reappears. The only possible source for the air is the tracheal extension. If the air be first removed from the tracheal extension, the filaments permanently lose their silvery appearance after the air has once been driven out by alcohol.

At the posterior end the extension is connected with the spiracle-chamber. At this point the chitinous intima loses its retiform thickening, and is developed into fibrillæ, which are similar to those that traverse the air-containing space in the gill. A perforated diaphragm is thus formed, which reduces the opening of the tracheal extension to a series of extremely minute apertures. No account of the meaning of this structure can be given.

From the preceding description it will be seen that the cavity of the gill is shut off from the cavity of

the body. Repeated examination of sections taken in various planes through the gill-base seems to make this point certain. It is also noticeable in these sections that the underlying epithelium is absent. In the developing gill in the larva, and for some time after pupation, the epithelium is present. It disappears eventually, and in preparations of old filaments presents a disorganised appearance. In an old pupa the filaments are frequently broken across, and it is not uncommon to find that foreign bodies, *e. g.* diatoms, have entered the cavity of the gill through the accidental opening. From these observations it seems likely that the interchange of gases between the gill and the surrounding water is carried on without the intervention of a cellular epithelium.

The respiratory organs of the pupa of Simulium have often been described as tracheal gills. Tracheæ, however, are quite absent, and Vogler (No. 6, p. 33) has therefore suggested the name *tube-gills* (*Röhrenkiemen*) for these structures. The filaments are certainly tubular; since, however, air does not occur within the cavity, but is confined to spaces excavated in the cuticular wall, it seems more appropriate to describe them as *cuticular gills*.

De Meijere (Nos. 1 and 2) has recently published accounts of the compound spiracles of Dipterous larvæ and pupæ. According to these observations, the original spiracle becomes closed, and the trachea leading from it to the adjacent tracheal trunk collapses and forms a solid cord. The closed spiracle De Meijere names the *outer stigmatic scar* (*äussere Stigmennarbe*); the solid cord, the *scar cord* (*Narbenstrang*), and the place where it joins the tracheal trunk the *inner stigmatic scar* (*innere Stigmennarbe*). The new larval or pupal spiracle arises as an outgrowth from the adjacent trachea close to the inner stigmatic scar. The outgrowth grows towards, and comes in contact with the skin. It becomes hollow, and contains a special chamber, the chitinous wall of which shows a retiform thickening, and is often clothed with a felt of hairs. This chamber De Meijere names the *felted chamber* (*Filzkammer*). At the place where the felted chamber becomes attached to the skin, the external cuticle shows thin spots which are named *pits* (*Tüpfel*), from their resemblance to the thin places in the walls of plant-cells. From the presence of these pits, the secondary spiracle is named a *pitted stigma*

(*Tüpfelstigma*). In the simplest case the stigmatic area on which the pits are grouped is flush with the cuticle. Generally, however, the pits are carried on one or more special budlike projections (*Knospen*) of the skin which contain prolongations of the felted chamber. In other cases the base of the pitted stigma is produced into a horn (*Prothorakalhorn*) which contains a continuation of the felted chamber, and carries the pitted stigma with its buds at the end. The distal portion of the felted chamber lying within the horn De Meijere distinguishes as the *felted chamber of the horn* (*Hornfilzkammer*) and the proximal part within the body as the *Narbenfilzkammer*. A further complication is brought about by the formation of a second pitted stigma on the felted chamber proximal to the original one. The original stigma may then be distinguished as the distal, and the second as the proximal pitted stigma. The old spiracle, now closed, and the adjacent new one together constitute what De Meijere calls the *compound spiracle*. The respiratory organ on each side of the thorax of Dipterous pupæ he regards as a prothoracic pitted stigma, differing from the similar organs of the larva and of the abdomen of the pupa only in its greater size. In rare cases the pitted stigma is absent and the respiratory function is performed by secondary structures (Chironomus).

From this account it will be seen that the gill-apparatus of the pupa of Simulium resembles the compound stigma of De Meijere in the following particulars: (1) The outer and inner stigmatic scars are connected by a solid cord (*Narbenstrang*, De Meijere; *initial thread*, Miall). (2) From that part of the tracheal trunk lying proximal to the inner stigmatic scar (*spiracle-chamber* of the present paper) a trachea with retiform markings (*Narbenfilzkammer*, De Meijere; *tracheal extension*, Miall) passes to the respiratory organ (*Prothorakalhorn*, De Meijere; *tracheal gill* of many writers; *tube-gill*, Vogler; *cuticular gill* of the present paper).

On the other hand the gill-apparatus of the Simulium-pupa differs from the compound stigma of De Meijere in the following respects: (1) The felted chamber of the horn is absent. (2) Stigma-pits and buds are absent. (3) The connection of the gill with the tracheal extension is made by means of a hollow cuticular ingrowth. From these observations there seems no reason to regard the

cuticular gill of the pupa of Simulium as a pitted stigma.

The development of the pupal gill begins in the young larva about the same time as that of the imaginal rudiments. It is noteworthy that this early origin of the gills in Simulium is not paralleled in Chironomus, where Miall and Hammond (No. 4, p. 124) find that "the dorsal prothoracic rudiments, from which the pupal tracheal gills of Chironomus proceed, are the last to be developed," and that it "is not till the larva is almost full-grown, and long after the other thoracic appendages are visible, that they appear." In the case of Simulium, the pupal gill and the imaginal rudiments make their appearance together. Of these, the dorsal prothoracic rudiment arises as an invagination of the epidermis, which takes place at the hinder end of the prothorax, immediately in front of the mesothoracic spiracle. The invagination gives rise to a shallow depression, lying at the side of the prothorax, whose wall becomes the sheath of the developing gill. The sheath, as seen from the outside, is semicircular in outline, with a straight and a curved margin. The straight margin runs obliquely upwards and forwards from the mesothoracic spiracle, and the rounded margin lies towards the anterior side. The wall bends sharply inwards along the straight edge, and is formed of a thickened epidermis. At its dorsal end an outgrowth of the thickened epidermis develops, and projects forwards and downwards into the cavity of the sheath as the rudiment of the gill. It soon divides more than once, and the branches become coiled up within the sheath. The gill-rudiment is hollow, and the cavity is continuous with the cavity of the body.

Dorsal to the gill-base and immediately behind the sheath a second invagination of the epidermis gives rise to a groove leading from the gill-base to the mesothoracic spiracle. The groove closes to form a tube (the tracheal extension), one end of which becomes continuous with the epithelium of the spiracle-chamber; the other ends blindly immediately below the gill-base. The epithelium of the tube secretes an intima on its free surface, which shows a retiform thickening. A thickening of the epithelium of the gill-base, which almost obliterates the cavity, grows inwards to meet the tracheal extension. The chitinous wall of the gill then becomes differentiated. The superficial layer with the fibrillæ forms first, and the homogeneous

deep layer later. As the cuticular wall develops it assumes a dark colour, which makes the gill a conspicuous object on the side of the thorax.


Fig. 6.

Bisection of Simulium-fly showing tracheal system. × 36.
*ll*, *ll*, longitudinal trunk; *d*, *d*, dorsal commissures; *sc*, mesothoracic spiracle-chamber; *sc'*, metathoracic do; *sp*, spiracle of 3rd abdominal segment.

When the larva is full-grown it weaves around its body a case, and then turns into a pupa. In *S. latipes* the whole

operation lasts about an hour, and requires considerable exertion, the larva bending its body in all directions as it pays out the silken thread. When the case is nearly completed, the larva pauses for a moment. The pupal extension suddenly fills with air, and becomes a conspicuous object on the side of the thorax. While the larva is putting the finishing touches to the pupal case, the air slowly creeps into the gill-base and along the filaments, which are still coiled up beneath the larval skin. It is an interesting spectacle to see the extension suddenly flash into view, and to watch the silvering of the filaments. When all is ready, the larval skin cracks, and the filaments, now filled with air, uncoil and project freely into the water flowing past.

The tracheal system of the fly (fig. 6) shows the same general arrangement as that of the pupa. The longitudinal trunks are connected by commissures, and the same branches with some additions are found as in the pupa. In the abdomen the posterior half of the longitudinal trunk and its ventral segmental branches give off many small tracheæ, which are unrepresented either in the pupa or larva. The tracheal extension of the pupa is unrepresented in the fly. The brushes of tracheolæ in the thorax of the pupa are replaced by vesicular tracheæ, and there is a general tendency, especially in the thorax, for the tracheæ to become dilated. In the abdomen open spiracles are present in segments 3—7 inclusive. Each consists of a small chamber with thickened chitinous walls of a dark colour, opening to the exterior, and also communicating by a short side-branch with the ventral segmental trachea. Spiracles have not been found in the first two segments of the abdomen. That they are present in some form may be inferred from the fact that in the pupa the tracheal remnants of these segments are withdrawn, and left attached to the cast skin when the fly has emerged. The thorax of the fly is provided with two pairs of spiracles, an anterior and a posterior. The position of these is not readily determined. It might be supposed that it would be safe to conclude that the homologous spiracle of the larva ought to afford an indication of the position of a spiracle in the fly. And if it could be shown that the generating epithelium was identical in the two spiracles, such evidence would carry weight. In the case of the anterior thoracic spiracles of Simulium, however, such an

identity cannot be demonstrated. The reverse seems to be inferred from the following considerations. In the larva the mesothoracic spiracle is connected with the spiracle-chamber by an initial thread. In the pupa the same arrangement is found, and in addition a tracheal extension connects the spiracle-chamber with the gill. In the fly it might have been supposed that the same arrangement as in the pupa would hold with these differences: (1) the tracheal extension would end blindly at the surface of the body in a scar marking the former insertion of the cast gill;

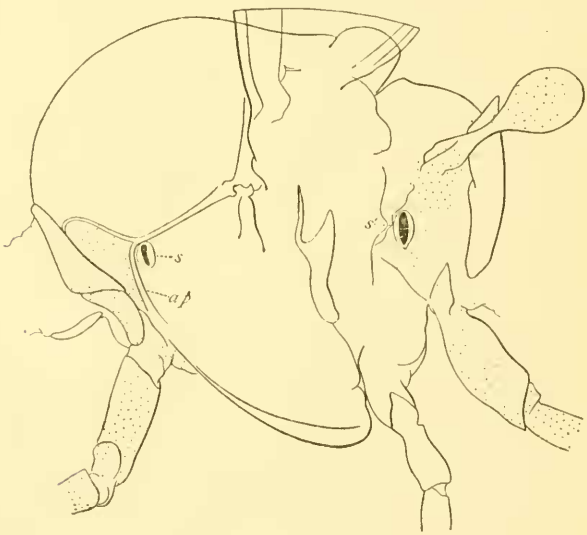


Fig. 7.

Thorax of Simulium-fly in side view. The pro- and metathorax with their appendages are dotted. × 45.

*s*, mesothoracic spiracle; *s'*, metathoracic do.; *ap*, apodeme marking junction of pro- and mesothorax.

(2) the spiracle would be open, and in communication with the spiracle-chamber by a hollow initial tube. The actual arrangement is, however, quite different. The epithelium of the initial thread and of the tracheal extension becomes flattened out, and takes part in the formation of the body-wall. The spiracle-chamber thus comes to lie close to the external surface, and the apertures of the initial thread and the extension coalesce to form the functional spiracle of the fly. The anterior thoracic spiracle of the fly is thus

a new formation, and does not correspond to the mesothoracic spiracle of the larva or of the pupa. The inquiry as to its position must therefore be made from data furnished by the fly itself.

Immediately in front of the anterior thoracic spiracle of the fly a well-marked apodeme (fig. 7, *ap*), or thickening of the cuticle, runs obliquely backwards to the mid-ventral line, where it unites with a similar thickening on the other side. The fore leg of the fly is inserted on the body in front of this apodeme, and muscles from the leg are attached to it. As the apodeme approaches the midventral line, it bears the antefurca, which, wherever it occurs, marks the junction of the pro- and mesothorax. The anterior spiracle of the thorax lies close behind the apodeme which carries the antefurca, and is therefore mesothoracic in position.

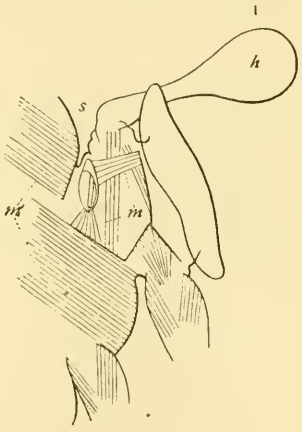


Fig. 8.

Vertical section of metathorax of fly. × 50.

*h*, haltere; *s*, spiracle with muscles, *m*; *m'*, mesothoracic muscles.

The posterior thoracic spiracle is formed out of the metathoracic spiracle-chamber of the pupa. It lies ventral to the haltere; immediately in front of it a deep suture occurs in the body-wall, which indicates the division between the meso- and metathorax. Further proof that this spiracle is metathoracic in position is afforded by sections. In fig. 8, which is constructed from sagittal sections, the muscles of the spiracle are seen arising from the same apodemes as the muscles of the haltere.

I desire to gratefully acknowledge my indebtedness to Professor Miall for proposing the subject of this paper, and for much help and kind interest during its preparation.

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